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Wolffiella neotropica, clone 8848

Cover page

Wolffiella neotropica, clone 8848

Wolffiella neotropica is a species endemic to South America, with only a limited number of clones collected from Brazil and Venezuela. Clone 8848 was from Rio de Janeiro, Brazil, and is currently being sequenced for construction of a reference genome (Michael and Lam Labs). K-mer analysis indicates a haploid genome size of about 500 Mbp, making it one of the smallest genome known for this genus. Like several other *Wolffiella* species, *We. neotropica* fronds display a saddle-like shape with both ends immersed under the surface of the water body. On agar plates, this frond morphology apparently can result in clusters made up of concentric "rings" of fronds that resemble bouquets, as depicted here. Photo credit: Prof. Dr. Eric Lam, Rutgers, The state University of NJ, New Brunswick, USA.

In this issue

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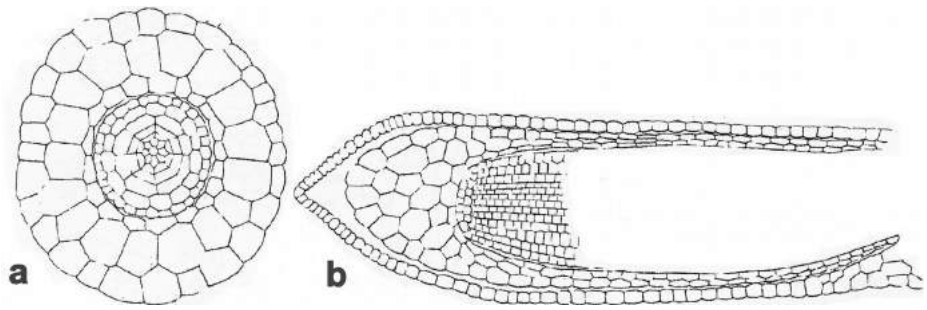
Science meets art: Anatomy of *Lemna minor L.*



Root morphology. a: Cross section through a root near the root tip with root cap and rhizodermis. b: Longitudinal section through a root (0.5 cm). Drawing by Christoph Friedrich Hegelmaier (1833-1906), In: Die Lemnaceen: Eine Monographische Untersuchung. Wilhelm Engelmann, Leipzig, 1868.

Letter from the Editor:

Dear Friends and Duckweed Researchers,

Another year has begun and I hope this new issue of our *Duckweed Forum* finds you well at your neck of the woods. As we begin 2021, we eagerly look forward to the proverbial "end of the tunnel" to take us out of the darkness that the COVID pendemic has shrouded humanity in for most of last year. While the speedy production of effective vaccines has been an amazing scientific achievement, it is still sobering and sad to note that more than 2 million people globally has perished so far, with still more in the coming months projected. This pandemic has illustrated how fragile our global ecosystem is and the disparity in access to proper medical treatment and ease of information manipulation for political gains are vividly demonstrated. As scientists and responsible entrepreneurs, it is ever more urgent that we maintain our effort to work together for the common good and help to educate the public about the importance of fact- and data-based approaches to problem solving and disaster prevention. The sharing of ideas and information between us is one of the key actions that can help propagate creative solutions as well as to break down barriers between disciplines and nationalities. This remains the objectives for our newsletter.

In this issue of the *Duckweed Forum*, we have an Historical Account of Prof. Kandeler by our ISCDRA member Klaus Appenroth. It was an interesting read for me since I did not know much about Prof. Kandeler except as a co-author with Prof. Landolt for one of the famed monographs for all-things-duckweed. After reading Klaus' account, I feel that I have a better appreciation of Prof. Kandeler's efforts and his circumstances when carrying out his work with duckweed in challenging times. Also, Klaus and his co-authors have contributed an instructive species identification key for duckweeds to lay out the step-by-step approach for newcomers to the duckweed field. Finally, a postdoctoral fellow in my lab, Buntora Pasaribu, is featured in the Student Spotlight section. As usual, Klaus has generously dispensed with his time and effort to summarize recently published scientific papers on duckweed in the Database section. It is always a great resource for me to quickly get an update on the "pulse" of the duckweed field and to appreciate how much diverse interest and applications that these little plants are continuing to generate all over the world.

Lastly, I like to acknowledge the numerous contributors for their time and effort to share interesting ideas and knowledge with others in our community. I hope many of you will think about approaching us to share your ideas with us and make your contribution in the near future. Good luck and be well in 2021!

Sincerely,

Eric Lam

Chair, ISCDRA

Update of the morphological key for identification of duckweed species

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In the last decade, significant progress in the identification of duckweed species has been made with the help of molecular tools such as amplified fragment length polymorphism (AFLP; Bog et al., 2019), DNA barcoding on the basis of selected plastidic sequences (Borisjuk et al., 2015; Bog et al. 2019) and other methods (Bog et al., 2020a, b; review in Bog et al., 2019). However, for at least two reasons, the molecular taxonomic methods that were used so far cannot satisfy all practical requirements: (1.) Still not all 36 duckweed species can be identified by molecular methods, with confidence. This holds true e.g. for distinguishing between the species *Lemna minor* and *L. japonica*. Further examples exist in the genera *Wolffia* (Bog et al., 2013) and *Wolffiella* (Bog et al., 2018). (2.) For practical reasons, molecular methods are not the first choice for all scientific questions. Best examples come from ecological and biodiversity studies. When one wants to investigate the duckweed species diversity of a certain water body or geographical region, then an initial morphology-based screening and segregation of the plant samples collected from a particular site is an essential exercise, for the fact that barcoding of a mixture of different related species (when morphology based sample collection is not performed) may result in improper identification. In cases when one encountered problems with the occurrence of morphological markers, or if researcher assumes the existence of an unexpected species within that region, e.g. species from tropical areas in temperate climates as consequence of global warming, e.g. *Lemna minuta* in Europe (Czechin et al., 2018; Paolacci et al., 2018) or several *Wolffia* species in Germany (Beigel, 2020; Frank et al., 2021), one would then move on with molecular methods, preferably DNA barcoding with established primer sets as a starting point.

For these reasons, the first approach to identify duckweed species is usually a morphological key that would help to at least narrow down the possibilities. Elias Landolt has published morphological keys to aide duckweed identification in the past (Landolt 1980, 1986). However, after decades of duckweed research, several species were discovered, re-named or united, thereby revising multiple times the number of recognized duckweed species (Sree et al., 2016).

Therefore, an updated "Key to the determination of Lemnaceae taxa" was urgently needed (Bog et al., 2020).

For beginners, usually the question arises where to start with, meaning which morphological marker should be examined first. To visualize this procedure, we are presenting the general structure of Lemnaceae in Fig. 1. Let's start with the question, whether there are roots or not? Duckweeds with roots belong to the subfamily Lemnoideae comprising the genera *Spirodela* (consisting of 2 species), *Landoltia* (only a single species) and *Lemna* (12 species). This subfamily is not a perfect taxon because it is polyphyletic (more than one common ancestor). Taxonomists try to avoid defining such groups as a taxon. Taxonomy is influenced by knowledge of evolution and tries to define taxa in agreement with it. However, as you can see, identification of "Lemnoideae" is a very useful step in the morphological identification, even when it is not monophyletic. If there are no roots, the duckweed species of interest belongs to the subfamily Wolffioideae comprising the genera *Wolffiella* (10 species) and *Wolffia* (11 species).

We may proceed further here in this short article to identify at least the genus of a species to be identified. In root-bearing fronds the number of roots is important. If there are normally 6 to 21 roots per frond, this speaks in favour of *Spirodela*. Here and in all other cases one has to take care to count the number of roots per single frond as several fronds together usually form a colony/cluster of fronds. Another useful sign is the fact that *S. polyrhiza*, in contrast to *S. intermedia*, can form turions, smaller green-brownish lens-shaped structures without roots (or only very small root-primordia) both in nature and in the laboratory. It should also be mentioned that the geographic occurrence may help to identify the species: *S. intermedia* exists mainly in warm, temperate, subtropical and tropical regions of South and Central America whereas *S. polyrhiza* can be found in almost all countries. The only species of *Landoltia*, *L. punctata* has normally 2 to 7 roots and has much smaller fronds than the two *Spirodela* species. It has almost the size and shape of a *Lemna*. A very good marker is the existence of a row of papillae on the upper surface of *L. punctata* fronds compared to the genus *Spirodela*. Some species of the genus *Lemna* also show these papillae, but can be easily distinguished from *Landoltia* since they have only one root per frond.

Duckweed species without roots belong to two genera in the second subfamily, *Wolffiella* and *Wolffia* (see Fig. 1). Species of *Wolffiella* have many different forms but are in general rather flat and often elongated fronds. They form sometimes unusual colonies, e.g. star-like. Fronds of the second genus, *Wolffia*, have in general ball-shaped, globular bodies. In a few cases, an appendix like structure may be present, e.g. the pseudo-root in *Wolffia microscopica* (Landolt et al. 1986; Sree et al., 2015).

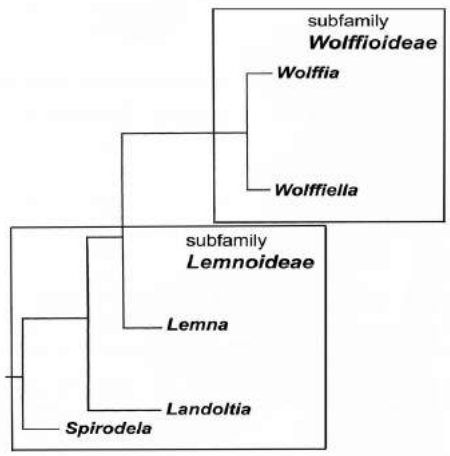

Figure 1: Family Lemnaceae

Now we have kick-started the morphological identification of duckweed species. To go further, the above-mentioned published key (Bog et al., 2020) should be consulted in terms of finer morphological and physiological traits that can help to distinguish species within a particular genera. We hope that this starter guideline is useful for newcomers to the duckweed field. Good success!

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Historical Account: Riklef Kandeler (1927 – 2015)

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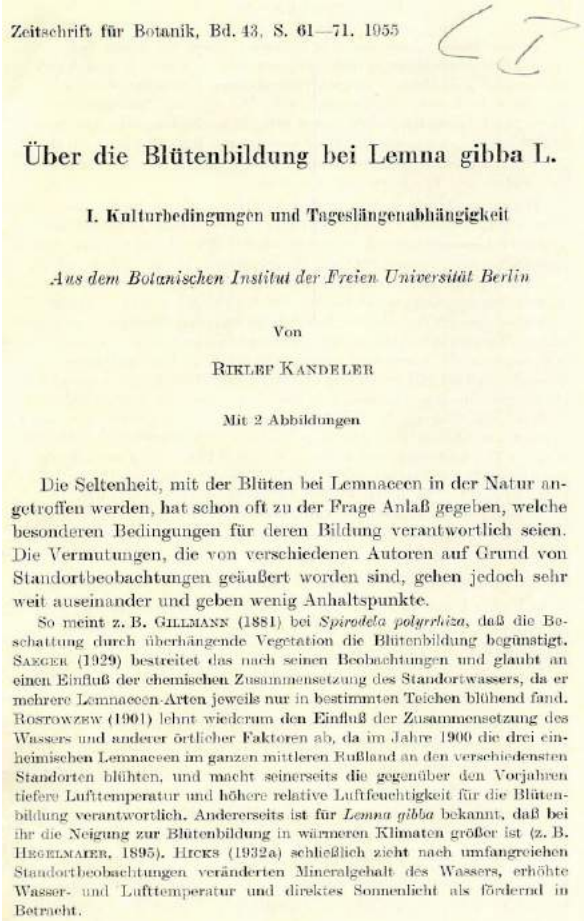
Riklef Kandeler was born on June 18th, 1927, in Berlin. After World War II he studied Biology at the Free University Berlin and in 1951 he received his PhD degree (Dr. rer. nat., *doctor rerum naturalium*) at the same University. Evidently, he already started during this time to investigate duckweeds, i.e. flower induction in *Lemna gibba* (Kandeler, 1955; Fig.1). Thereafter, he moved to the Julius-Maximilian-University in Würzburg. There he became habilitated (Dr. habil., *doctor habilitatus*) in 1960 with the topic "About the light-dependence of anthocyanin formation" (Kandeler, 1960). He told me that he ran into scientific conflicts with some leading botanists in Germany on the role of phytohormones in photomorphogenesis and preferred to move to Vienna, Austria. In 1972 he received a call to the University of Natural Resources and Life Sciences in Vienna (Universität fuer Bodenkultur, BOKU). He became Director of the Institute of Botany there until his retirement in 1993 (Fig.2). He passed away on March 22, 2015. His grave is located in the Forest Cemetery at Würzburg.



Zeitschrift für Botanik, Bd. 43, S. 61—71. 1955

Über die Blütenbildung bei Lemna gibba L.

I. Kulturbedingungen und Tageslängenabhängigkeit

Aus dem Botanischen Institut der Freien Universität Berlin

Von

RIKLEF KANDELER

Mit 2 Abbildungen

Die Seltenheit, mit der Blüten bei Lemnaceen in der Natur angetroffen werden, hat schon oft zu der Frage Anlaß gegeben, welche besonderen Bedingungen für deren Bildung verantwortlich seien. Die Vermutungen, die von verschiedenen Autoren auf Grund von Standortbeobachtungen geäußert worden sind, gehen jedoch sehr weit auseinander und geben wenig Anhaltspunkte.

So meint z. B. GILLMANN (1881) bei *Spirodela polyrrhiza*, daß die Beschattung durch überhängende Vegetation die Blütenbildung begünstigt. SAEGER (1929) bestreitet das nach seinen Beobachtungen und glaubt an einen Einfluß der chemischen Zusammensetzung des Standortwassers, da er mehrere Lemnaceen-Arten jeweils nur in bestimmten Teichen blühend fand. ROSTOWZEW (1901) lehnt wiederum den Einfluß der Zusammensetzung des Wassers und anderer örtlicher Faktoren ab, da im Jahre 1900 die drei einheimischen Lemnaceen im ganzen mittleren Rußland an den verschiedensten Standorten blühten, und macht seinerseits die gegenüber den Vorjahren tiefere Lufttemperatur und höhere relative Luftfeuchtigkeit für die Blütenbildung verantwortlich. Andererseits ist für *Lemna gibba* bekannt, daß bei ihr die Neigung zur Blütenbildung in wärmeren Klimaten größer ist (z. B. HEGELMAIER, 1895). HICKS (1932a) schließlich zieht nach umfangreichen Standortbeobachtungen veränderten Mineralgehalt des Wassers, erhöhte Wasser- und Lufttemperatur und direktes Sonnenlicht als fördernd in Betracht.

Fig. 1 (to the left): First page of the first publication of Riklef Kandeler about duckweed.

Fig. 2 (above): Professor Kandeler (third from left) during the celebration of his 80th birthday in July 2007 at the University of Natural Resources and Life Sciences in Vienna.

Professor Riklef Kandeler was mainly interested in developmental processes of plants and used duckweeds (Lemnaceae) as model system. In his axenic stock collection were at least 13 clones of *Lemna gibba*, labelled as G1 to G13 (Kandeler, 1975). He collected the clone G1 himself in Berlin-

Schildow (Tegeler Fliess) but this clone got lost in 2011 and intensive search for it, even with the help of the German Botanical Society, was not successful. Kandeler described to the present author the precise place of origin but several strains collected there turned out to be *Lemna minor* as shown by molecular barcoding. More famous is the clone *L. gibba* G3, which was provided by K. R. Appel, collected in the Botanical garden of Catania, Sicilia (Kandeler, 1969) but evidently isolated (sterilised) by Kandeler. He wrote to me that he learned to sterilize duckweeds from Andre Pirson (see the related article in Duckweed Forum 8: 100-101 (2020)). The clone G3 was cultivated by Kandeler since 1953 under axenic culture conditions and is still intensively used for standardised phytotoxicity investigations, especially according to the OECD 221 protocol.

Kandeler investigated much the effect of different external factors on flower induction in several duckweed species. He investigated day length (Kandeler, 1955, 1962), produced an action spectrum using low light fluence (Kandeler, 1956), the influence of photosynthesis (Kandeler, 1971b; Schuster and Kandeler, 1970; Kandeler et al., 1974) and the effects of many chemical substances such as polyamine (Decantu and Kandeler, 1989), CO_2 and bicarbonate (Kandeler, 1964a, b), ammonium (Kandeler 1969a), DCMU, Lithium and ATP (Kandeler, 1969b, 1970), ascorbic acid, NADH and NADPH (Kandeler 1971b), acetylcholine (Kandeler, 1972), abscisic acid and the gibberellic acid inhibitor chlorcholinchlorid (Kandeler and Huegel, 1973), as well as EDDHA and salicylic acid (Scharfetter et al., 1978). He could show that senescence has opposite effects on flowering induction in *Lemna aequinoctialis* (synonym *L. paucicostata*) and *L. gibba* (Kandeler et al., 1974). These results were summarized by Kandeler (1968, 1984). Unfortunately, in that time no molecular tool existed that could be use to investigate the mechanisms of the described effects. The situation has now changed completely and some of the effects deserve to be re-investigated.

Kandeler was very much interested in effects of the photoreceptor phytochrome. He investigated the role of phytochrome on turion formation in *Lemna perpusilla* (Huegel et al., 1979), on vegetative growth of *L. gibba* (Kandeler, 1963, 1966) and on the so-called effect of overcrowding. When the surface is covered by fronds so much that fronds have to touch each other (overcrowding), plants decrease their growth rates. Kandeler could show that this effect depends on phytochrome, that the intracellular Ca^{2+}-concentration increased transiently, followed by emission of ethylene, which is known to inhibit growth of duckweeds (Farber et al., 1986; Farber and Kandeler, 1989, 1990). Hartung and Kandeler (1976) discovered that the transport of labelled phytohormones is regulated by the so-called end-of-day far red irradiation. He summarized the role of phytohormones in photomorphogenesis and this brought him to one of his most important publication (Kandeler, 1974).

Although Kandeler was basically a plant physiologist, he also worked as a taxonomist. He re-discovered the real duckweed species *L. perpusilla*, which was combined for some time erroneously with *Lemna aequinoctialis* (Kandeler and Huegel, 1974). Moreover, he showed the broad phenotypic variation of *Lemna minor*, which makes differentiation between this species and *Lemna gibba* sometimes very difficult (Kandeler, 1975). Whereas the delineation between the two species is no problem for molecular barcoding anymore, the large phenotypic variation in *L. minor* clones still awaits more detailed investigation at the genomic level.

Kandeler wrote several articles to give an overview about the family of duckweed and help to popularize it (Kandeler, 1961, 1979, 1985, 1988). Together with Elias Landolt, he probably wrote his most important book "Biosystematic investigations in the family of duckweed (Lemnaceae); Vol. 4" (Landolt and Kandeler, 1987). This book, together with the other volume written by E. Landolt (Landolt, 1986) remains indispensable to this date for scientists entering the field of duckweed.

After his retirement, Professor Kandeler focused mainly on the interaction of Botany and Art and the symbolic meanings of plants and colours in paintings and mosaics (Fig. 3; Kandeler, 1987, 2003; Kandeler and Ullrich, 2009). He was especially interested in the so-called "Blue Flower", a symbol for happiness from the time of the Romantic, represented e.g. by Novalis (Georg Philipp Friedrich von Hardenberg, 1772-1801). Novalis studied Law in Jena from 1790 to 1794. Riklef Kandeler searched intensively in Jena for the existence and botanical identity of the "Blue Flower", unfortunately without

success. However, this was not the only reason why he felt himself connected with Jena and visited the University several times. He kept intense contact with Professor Helmut Augsten in Jena, who was director of the Department of Plant Physiology and with a main research focus on duckweed (Fig. 4).

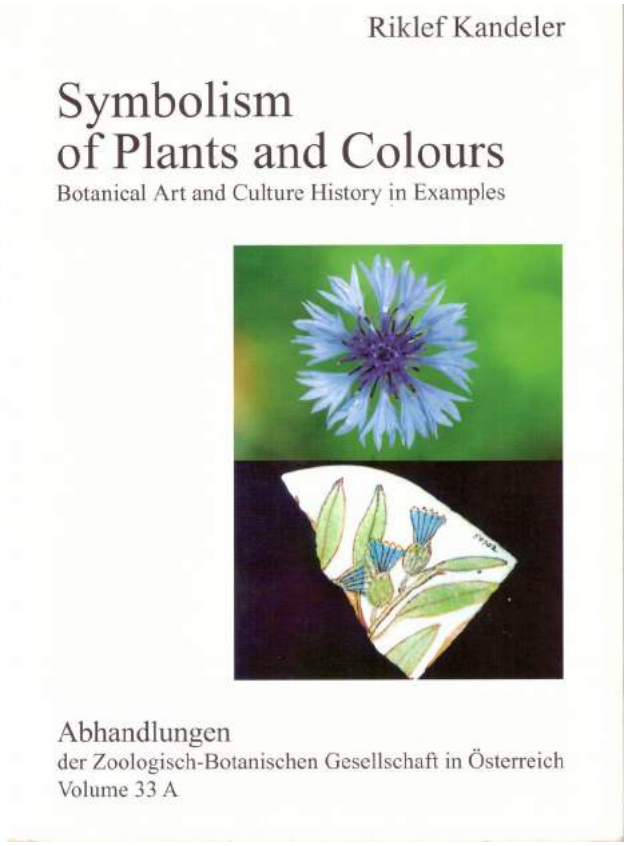


Fig. 3 (to the left): Cover page of the last publication of Riklef Kandeler, Vienna 2003.

Fig. 4 (above): Professor Kandeler during a lecture at the University of Jena, Department of Plant Physiology on the occasion of the 60th birthday of Professor Helmut Augsten in 1988 about the topic "Survival Strategies of duckweeds" (see also Kandeler, 1988).

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Student Spotlight: Buntora Pasaribu

Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, New Jersey, USA (Email: buntora86@gmail.com)

Since I was born in the archipelago country of Indonesia, I do believe the marine/aquatic environment is a part of my life. I grew up in a small county call Bengkalis, with Riau as the nearest city, where my house was located near the edge of the ocean. In college, I earned a Bachelor's degree in Fisheries Technology, which speaks to how I feel about aquatic life. Since joining a project to establish an algae system for CO_2 sequestration in Indonesia, I have been amazed by algal strains that are able to increase their oil content rapidly upon stress. This trait makes algae a promising candidate as a bioenergy feedstock to replace petroleum in the future. I am also fascinated with the ability of plants to deposit stores of carbohydrates, proteins, and neutral lipids in subcellular particles such as starch granules, protein bodies, and oil bodies/lipid droplets, respectively. For continuation of my research career, I had an opportunity to pursue my master's degree in Marine Biotechnology (National Dong Hwa University, Pingtung, Taiwan) and worked on mutualism association that occur between coral and algae/*Symbiodinium* sp. I was trained at the National Museum of Marine Biology and Aquarium, which is a top-tier research institute in marine biology and boasts the largest marine aquarium in Taiwan. I investigated the impact of environmental stresses (nutrients and temperature) on morphology and lipid biogenesis in coral and algae/*Symbiodinium* sp. and developed a method to isolate and purify lipid droplets from endosymbiotic *Symbiodinium* sp. cells. I observed that a novel protein related to caleosin (one of the integral proteins found in plants) is associated with the endosymbiotic *Symbiodinium* sp. lipid droplets in coral and named as "SLDP" (*Symbiodinium* sp. Lipid Droplets Protein). My research passion has guided me to pursue my doctoral study in Biotechnology (National Chung Hsing University, Taichung, Taiwan) under the supervision of Dr. Jason Tzen. During my PhD, I was challenged to study land plants, for which I did not have a solid background. However, I managed to discover many interesting scientific findings from my study. I investigated vascular plants in the Pinaceae family believed to have existed in the Triassic period (around 245–208 million years ago) and observed that numerous oil bodies occupied approximately 80% of the intracellular space in those vascular plants. These were isolated and found to comprise of mostly triacylglycerols. Complete cDNA fragments encoding three pine oil-body proteins, tentatively named caleosin, oleosin-L, and oleosin-G were obtained and sequenced.

Fig 1. Harvesting *Lemna minor* in the greenhouse

My aquatic research journey then continued at Rutgers University. I could say that I was fortunate to be able to join Dr. Eric Lam's lab (Rutgers University, New Brunswick, USA). I do remember a question being asked by Dr. Lam during my interview as to whether I know about "duckweed." These remarkable aquatic plants amaze me with their potential benefits. I started my work as a postdoctoral fellow and it appears to be a tradition in the Lam lab to taste duckweed before you work with them, as I quote the saying: "If you never try, you will never know". I was trained to maintain them (Fig. 1) and was introduced to many duckweed clones collected from around the world. With guidance from Dr. Eric Lam, a duckweed expert, and access to the Rutgers Duckweed Stock Cooperative (RDSC), the largest collection of living duckweed

strains in the world, I was able to appreciate the broad view of duckweed-omics. My main project is to investigate the molecular mechanism of turion induction in *Spirodela polyrhiza* with phosphate limitation as the most well-established environmental signal. *Sp. polyrhiza*, commonly known as "Greater Duckweed," exhibits a prominent survival behavior under conditions of abiotic stresses. In unfavorable environments, such as low temperature, low light, and nutrient limitation, clones of *Sp. polyrhiza* and some others in the duckweed family are able to differentiate their frond meristem to form a novel dormant structure that detaches from the mother frond.

This so-called "turion" (Fig. 2) contains high starch content and, upon detachment, sinks to the bottom of the water body to await the return of appropriate conditions for germination and growth. While some of the factors that affect this remarkable survival strategy are known, little has been clarified at the molecular and mechanistic levels. One of the major challenge is the difficulty in isolating high quality nucleic acids, especially RNA, from turions due in part to their high starch content (>70% dry weight). To overcome this key bottleneck, I treated Sp clones under low phosphate condition to induce turion formation and carefully harvested mature turions from the bottom of the baby jars. Then, the mature turions were immediately soak in liquid nitrogen and stored in a -80°C freezer (for long term usage). This allows one to have a supply of fresh turion samples for future RNA isolation. I found that disruption of the mature turion cells determines the success rate of high-quality RNA isolation from turions. Therefore, this first step required more systematic optimization. I examined several approaches to disrupt the mature turions whether by using a bead beater or a mortar and pestle. I found that the combination of both methods is the optimal approach, resulting in the best RNA yield and quality. Currently, I am using RNA-seq approach to mine the candidate genes and potential regulators for turion induction and biogenesis. Through identification of the turion regulators, it could enable us to understand the biological changes during developmental transition in duckweed.

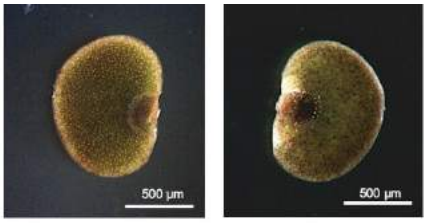

Fig.2: Turion of *Spirodela polyrhiza* 9509: Left – ventral side, Right– dorsal side

Last year, I was given an opportunity to present my work in the Plant and Animal Genomics XXVIII, at San Diego, in 2020 (Fig. 3). I was able to meet duckweed researchers from different countries and received constructive comments for my study. Duckweed will always amaze me with their biological system and potential benefits. Therefore, I will keep exploring the scientific question for this model plant.

Fig. 3. Attending the Plant and Animal Genomics XXVIII at San Diego, USA

Update of duckweed stock collections

The following are the up-to-date details of duckweed stock collections available worldwide in accordance with the newly introduced duckweed clone registration system (ISCDRA 2020).

Table: Up-to-date details of Duckweed Stock Collections across the globe.

S. No.	Director of the stock collection (Email ID)	Affiliation	Clone ID in individual system	Available clones with Landolt ID system	Available clones with individual ID system
1	Klaus J. Appenroth, Klaus.Appenroth@uni-jena.de	Matthias Schleiden Institute - Plant Physiology, University of Jena, Jena, Germany	KJAxxx	550 live, 36 herbarium specimens	50 (with KSS and BOG)
2	Manuela Bog, manuela.bog@uni-greifswald.de	Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany	BOGxxx	200	100 (with KJA)
3	Nikolai Borisjuk, nborisjuk@yahoo.com	Jiangsu Key Lab Ecoagr Biotechnol Hongze Lake, Huaian, China	NBxxxx	11	24
4	Hongwei Hou, houhw@ihb.ac.cn	Chinese Acad Sci, Inst Hydrobiology, Wuhan, Hubei, China	HHWxxx	14	5
5	Marcel Jansen, M.Jansen@ucc.ie	University College Cork, Earth and Environmental Sciences, Cork, Ireland	MJxxx	25	will be created
6	Jitendra P. Khurana, khuranaj@genomeindia.org	Dept. of Plant Molecular Biology, University of Delhi, South Campus, New Delhi, India	JPKxxx	12	8
7	Walter Laemmler, wlaemmler@duckweed.ch	Landolt Stock Collection, Zurich, Switzerland	only Landolt numbers	400 live, 5000 herbarium specimens	
8	Robert Laird, robert.laird@uleth.ca	Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada	RLxxx	5	70 (with RDSC and CCPCC)

9	Eric Lam, ericl89@hotmail.com	Rutgers Duckweed Stock Cooperative (RDSC), Rutgers University, NY, USA	ELxxx	740	50
10	Massimiliano Lauria, lauria@ibba.cnr.it Laura Morello, morello@ibba.cnr.it	Institute of Agricultural Biology and Biotechnology; National Research Council, Milan, Italy	LMxxxx	85	11
11	Yubin Ma, mayubin@ouc.edu.cn	Ocean University of China, Qingdao, China	MYBxxxx	103	40
12	Viktor Olah, olahviktor@unideb.hu	Institute of Biology and Ecology University of Debrecen, Debrecen, Hungary	UDxxxx	7	34 (with MJ)
13	Ingo Schubert, schubert@ipk-gatersleben.de	Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany	only Landolt numbers	50	
14	K. Sowjanya Sree, ksowsree@gmail.com	Dept. of Environmental Science, Central University of Kerala, Periye, Kerala, India	KSSxxx	45 live, 36 herbarium specimens	27
15	Shuqing Xu, shuqing.xu@uni-muenster.de	Institute for Evolution and Biodiversity, University of Münster, Münster, Germany	XUxxx	150-160	5
16	Jiaming Zhang, zhangjiaming@itbb.org.cn	Institute of Tropical Bioscience and Biotechnology, Hainan, Haiku, China	ZJxxxx	50	700
17	Hai Zhao, zhaohai@cib.ac.cn	Chengdu Institute of Biology, Chinese Acad Sciences, Chengdu, China	ZHxxxx	640	150

Reference:

ISCDRA. 2020. Registration of duckweed clones/strains. *Duckweed Forum* 8: 35-37

From the Database

Highlight

Genome and time-of-day transcriptome of *Wolffia australiana* link morphological minimization with gene loss and less growth control

Michael, TP; Ernst, E; Hartwick, N; Chu, P; Bryant, D; Gilbert, S; Ortleb, S; Baggs, EL; Sree, KS; Appenroth, KJ; Fuchs, J; Jupe, F; Sandoval, JP; Krasileva, KV; Borisjuk, L; Mockler, TC; Ecker, J; Martienssen, RA; Lam, E (2020) Genome research DOI:10.1101/gr.266429.120

Rootless plants in the genus *Wolffia* are some of the fastest growing known plant on Earth. *Wolffia* have a reduced body plan, primarily multiplying through a budding-type of asexual reproduction. Here we generated draft reference genomes for *Wolffia australiana* (Benth.) Hartog & Plas, which has the smallest genome size in the genus at 357 Mb and has a reduced set of predicted protein-coding genes at about 15,000. Comparison between multiple high-quality draft genome sequences from *W. australiana* clones confirmed loss of several hundred genes that are highly conserved amongst flowering plants, including genes involved in root developmental and light signaling pathways. *Wolffia* has also lost most of the conserved NLR genes that are known to be involved in innate immunity, as well as those involved in terpene biosynthesis, while having a significant overrepresentation of genes in the sphingolipid pathways that may signify an alternative defense system. Diurnal expression analysis revealed that only 13% of *Wolffia* genes are expressed in a time-of-day (TOD) fashion, which is less than the typical ~40% found in several model plants under the same condition. In contrast to the model plants *Arabidopsis* and rice, many of the pathways associated with multi-cellular and developmental processes are not under TOD control in *W. australiana*, where genes that cycle the condition tested predominantly have carbon processing and chloroplast-related functions. The *Wolffia* genome and TOD expression dataset thus provide insight into the interplay between a streamlined plant body plan and optimized growth.

Chromosome-scale genome assembly for the duckweed *Spirodela intermedia*, integrating cytogenetic maps, PacBio and Oxford Nanopore libraries

Hoang, PTN; Fiebig, A; Novak, P; Macas, J; Cao, HX; Stepanenko, A; Chen, GM; Borisjuk, N; Scholz, U; Schubert, I (2020) Scientific Report 10: 19230

Duckweeds are small, free-floating, morphologically highly reduced organisms belonging to the monocot order Alismatales. They display the most rapid growth among flowering plants, vary similar to 14-fold in genome size and comprise five genera. *Spirodela* is the phylogenetically oldest genus with only two mainly asexually propagating species: *S. polyrhiza* (2n = 40; 160 Mbp/1C) and *S. intermedia* (2n = 36; 160 Mbp/1C). This study combined comparative cytogenetics and de novo genome assembly based on PacBio, Illumina and Oxford Nanopore (ON) reads to obtain the first genome reference for *S. intermedia* and to compare its genomic features with those of the sister species *S. polyrhiza*. Both species' genomes revealed little more than 20,000 putative protein-coding genes, very low rDNA copy numbers and a low amount of repetitive sequences, mainly Ty3/gypsy retroelements. The detection of a few new small chromosome rearrangements between both *Spirodela* species refined the karyotype and the chromosomal sequence assignment for *S. intermedia*.

Biotechnology

Production of Sorbents from Residual Biomass of *Chlorella sorokiniana* Microalgae and *Lemna minor* Duckweed

Politaeva, NA; Smyatskaya, YA; Efremova, SY (2020) Chemical and Petroleum Engineering 56: 543-547

The paper considers technologies for obtaining sorption materials from agricultural processing waste. It is shown that after the separation of valuable components from microalgae and duckweed a large amount of residual biomass is formed, which is a waste. This waste contains cellulose (23.5%), starch, hemicellulose, chitin-and pectin-like substances that cause the sorption properties of biomass. A microstructural analysis of the residual biomass showed a loose surface composed of the tissues of destroyed cells, which proves the high sorption capacity of the residual biomass. The possibility of using residual biomass for wastewater treatment to remove heavy metal ions has been studied and it has been shown that the maximum efficiency of treatment is achieved by residual biomass after heat treatment at 400°C. A unit for obtaining adsorption materials from residual biomass has been developed and created. Economic calculations of the developed installation were made, the payback period is 6 months, and the profit from using this setup is estimated to be $7899 per year.

High saccharification, low lignin, and high sustainability potential make duckweeds adequate as bioenergy feedstocks

Pagliuso, D; Grandis, A; Lam, E; Buckeridge, MS (2020) Bioenergy Research DOI: 10.1007/s12155-020-10211-x. Early Access: NOV 2020

Duckweeds are the smallest free-floating aquatic monocots. They have a unique cell wall containing pectin polymers named apiogalacturonan and xylogalacturonan. Knowing that the cell wall composition is essential for duckweeds as a bioenergy feedstock, notably ethanol production, this work reports the five duckweed species' (*Spirodela polyrhiza, Landoltia punctata, Lemna gibba, Wolffiella caudata*, and *Wolffia borealis*) composition and saccharification potential. Nonstructural carbohydrates were, on average, 42% of the dry weight. The cell wall comprises 20.1% pectin and glucomannan, 35.2% hemicelluloses, 30% cellulose, and 5% lignin, and the fermentable sugars in duckweed walls are glucose, galactose, and xylose. Together, these monosaccharides constitute 51.4% of the cell wall. Duckweeds displayed low recalcitrance to hydrolysis, probably due to the low lignin and cellulose contents. Furthermore, the saccharification of the duckweeds was higher than sugarcane, a primary bioethanol feedstock. Results indicate that duckweed biomass displays a high potential as a feedstock for bioethanol production.

A submerged duckweed mutant with abundant starch accumulation for bioethanol production

Liu, Y; Xu, H; Wang, Y; Tang, XF; He, G; Wang, SM; Ma, YB; Kong, YZ; Yu, CJ; Zhou, GK (2020) Global Change Biology Bioenergy 12: 1078-1091

Duckweed is one kind of promising bioenergy plant with prominent advantages such as fast growth rate and high starch content. However, almost all previous studies focused on the natural duckweed germplasms. In this study, heavy-ion irradiation was used to establish a mutant library of *Lemna aequinoctialis* 6002, and one mutant named submarine-1 (sub-1) was screened, which could accumulate more starch but with smaller granules. Unexpectedly, under proper external growth

conditions such as poor nutritional status and insufficient growth space, sub-1 mutant would sink underwater due to formation of dense tissue structure and large amount of fine starch particles with the extension of cultivation time. The starch content in the sinking sub-1increased to over 45% (dry weight) and was 12% higher than the floating sub-1, highlighting that submergence can be considered as a spontaneous and efficient indicator for screening of high-starch duckweed. Additionally, the saccharification efficiency of starch and ethanol yield had increased in sub-1mutant compared to the wild type. Based on the unique characteristics of sub-1 mutant, a cultivation model of submerged duckweed in a simulated aquaculture pond was designed to get more starch-rich biomass, enabling effective production of renewable bioenergy.

Duckweed from a biorefinery system: Nutrient recovery efficiency and forage value

Sonta, M; Lozicki, A; Szymanska, M; Sosulski, T; Szara, E; Was, A; van Pruissen, GWP; Cornelissen, RL (2020) Energies 13: 5261

This paper presents the results of an interdisciplinary study aimed at assessing the possibility of using duckweed to purify and recover nutrients from the effluent remaining after struvite precipitation and ammonia stripping from a liquid fraction of anaerobic digestate in a biorefinery located at a Dutch dairy cattle production farm. The nutritional value of duckweed obtained in a biorefinery was assessed as well. Duckweed (*Lemna minuta*) was cultured on a growth medium with various concentrations of effluent from a biorefinery (EFL) and digested slurry (DS) not subjected to the nutrient recovery process. The study's results showed that duckweed culture on the media with high contents of DS or EFL was impossible because they both inhibited its growth. After 15 days of culture, the highest duckweed yield was obtained from the ponds with DS or EFL contents in the medium reaching 0.39% (37.8 g fresh matter (FM) and 16.8 g FM per 8500 mL of the growth medium, respectively). The recovery of N by duckweed was approximately 75% and 81%, whereas that of P was approximately 45% and 55% of the growth media with EFL0.39% and DS0.39%, respectively. Duckweed obtained from the biorefinery proved to be a valuable high-protein feedstuff with high contents of alpha-tocopherol and carotenoids. With protein content in duckweed approximating 35.4-36.1%, it is possible to obtain 2-4 t of protein per 1 ha from EFL0.39% and DS0.39% ponds, respectively.

Ecology

Comparative ecotoxicity of single and binary mixtures exposures of cadmium and nickel on growth and biomarkers of *Lemna gibba*

Martinez, S; Saenz, ME; Alberdi, JL; Di Marzio, WD (2020) Ecotoxicology
DOI: 10.1007/s10646-020-02312-2; Early Access: NOV 2020

The aim of the present study was to investigate the toxicity effects of cadmium-nickel (Cd-Ni) after single and mixtures exposures over the macrophyte *Lemna gibba*. Effects were assessed on growth, as frond number and fresh weight and biochemical parameters, such as total protein content and activity of antioxidant enzymes. Plants were exposed to single Cd and Ni in concentrations that ranged between 0.13-33 mg/L and 0.18 and 11.82 mg/L, respectively. For binary mixtures, individual metal IC_{50} values were used for selection of the evaluated concentrations. The experimental design consisted in three different ratios based on the concept of toxic units (TU), each ratio was evaluated by five different concentrations. Both single and mixture treatments were performed for 7 days following the conditions according to OECD (2006). Single and mixture exposures affected plant growth and the biomarkers of the antioxidant response. Growth parameters showed a differential sensitivity after individual metal exposures. Cd was more toxic for *L. gibba* plants when fresh weight was considered, but on the contrary, considering frond number, Ni was the most toxic metal. IC_{50}-7d,

based on growth rate calculated on frond number were 17.8 and 2.47 mg/L, and on fresh weight were 1.08 and 3.89 mg/L, for Cd and Ni respectively. LOEC values for Cd were obtained at 2.06 and 1.03 mg/L, for frond number and fresh weight, respectively; while for Ni, these values were 0.92 and 11.82 mg/L. The three evaluated ratios for binary mixtures resulted in a high toxicity considering the same response variables in single metal exposures. Ratio 1 (2/3 TU Cd-1/3 TU Ni) was the most toxic considering both frond number and fresh weight, showing percentage inhibition of growth rates of 96 and 90%, respectively for the highest concentration. A modification of the protein content was observed in single, but especially in the mixture treatments. The activity of catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APOX; EC 1.11.1.11) and guiacol peroxidase (GPOX; EC 1.11.1.7) was also affected in single and mixtures assays. APOX and GPOX showed a higher increase of its activities respect the controls after mixture treatments than for single metal treatments. Such optimization of the antioxidant system could be one of the causes of the antagonistic toxicity observed in mixture exposures. Concentration addition (CA) reference model, based on frond number, in Cd-Ni mixtures was not a good predictor to evaluate toxicity from dissolved metal concentration since the results showed that toxicity was less than additive, with an average of sigma TU = 2.17. The observed antagonisms resulted to be stronger in mixtures with higher metal concentrations.

Feed & Food

Wolffia globosa-Mankai plant-based protein contains bioactive vitamin B-12 and is well absorbed in humans

Sela, I; Meir, AY; Brandis, A; Krajmalnik-Brown, R; Zeibich, L; Chang, DB; Dirks, B; Tsaban, G; Kaplan, A; Rinott, E; ; Zelicha, H; Arinos, S; Ceglarek, U; Isermann, B; Lapidot, M; Green, R; Shai, I (2020) Nutrients 12: 3067

Rare plants that contain corrinoid compounds mostly comprise cobalamin analogues, which may compete with cobalamin (vitamin B-12 (B-12)) metabolism. We examined the presence of B-12 in a cultivated strain of an aquatic plant: *Wolffia globosa* (Mankai), and predicted functional pathways using gut-bioreactor, and the effects of long-term Mankai consumption as a partial meat substitute, on serum B-12 concentrations. We used microbiological assay, liquid-chromatography/electrospray-ionization-tandem-mass-spectrometry (LC-MS/MS), and anoxic bioreactors for the B-12 experiments. We explored the effect of a green Mediterranean/low-meat diet, containing 100 g of frozen Mankai shake/day, on serum B-12 levels during the 18-month DIRECT-PLUS (ID:NCT03020186) weight-loss trial, compared with control and Mediterranean diet groups. The B-12 content of Mankai was consistent at different seasons ($p = 0.76$). Several cobalamin congeners (Hydroxocobalamin(OH-B-12); 5-deoxyadenosylcobalamin(Ado-B-12); methylcobalamin(Me-B-12); cyanocobalamin(CN-B-12)) were identified in Mankai extracts, whereas no pseudo B-12 was detected. A higher abundance of 16S-rRNA gene amplicon sequences associated with a genome containing a KEGG ortholog involved in microbial B-12 metabolism were observed, compared with control bioreactors that lacked Mankai. Following the DIRECT-PLUS intervention ($n = 294$ participants; retention-rate = 89%; baseline B-12 = 420.5 ± 187.8 pg/mL), serum B-12 increased by 5.2% in control, 9.9% in Mediterranean, and 15.4% in Mankai-containing green Mediterranean/low-meat diets ($p = 0.025$ between extreme groups). Mankai plant contains bioactive B-12 compounds and could serve as a B-12 plant-based food source.

Interaction with other organisms

Antibacterial activity of *Lemna minor* extracts against *Pseudomonas fluorescens* and safety evaluation in a zebrafish model

Gonzalez-Renteria, M; Del Carmen Monroy-Dosta, M; Guzman-Garcia, X; Hernandez-Calderas, I; Ramos-Lopez, YMA (2020) Saudi Journal of Biological Sciences 27:3465-3473

The treatment of bacterial diseases in aquaculture is done using antibiotics, their applications has resulted in contamination and bacterial resistance. Natural extracts are a potential alternative as an antimicrobial, they have demonstrated effectiveness in their use aimed at treating conditions. The purpose of this study was to evaluate the antimicrobial activity of *Lemna minor* extracts against *Pseudomonas fluorescens* with different solvent for extraction. Methanol, chloroform and hexane were used. Subsequently, the safety assessment of the extracts in *Danio rerio* embryos and larvae was performed to validate as ecologically harmless. Antibacterial activity was detected in three extracts with significant differences (p=0.001). Hexane extract had the highest antibacterial activity, followed by chloroform and methanol extracts. The three extracts have differences with respect to the control, between times and concentrations tested (p=0.001). Minimum inhibitory concentration values (MIC) at 24 h methanolic extract ME 0.05gm L^{-1}. In embryos and larvae increased safety of the LC_{50} methanolic extract was evidenced followed by the hexane and chloroform extract. No morphological or tissue changes were observed in embryos and larvae. The hexane extracts of *L. minor* had a greater bactericidal effect against *P. fluorescens* and are functional because of their antibacterial activity, but methanolic extract is more safety in embryos and larvae of *D. rerio*, making it a potential alternative for use in the treatment and control of septicemia in fish.

The duckweed, *Lemna minor* modulates heavy metal-induced oxidative stress in the Nile Tilapia, *Oreochromis niloticus*

Abdel-Gawad, FK; Khalil, WKB; Bassem, SM; Kumar, V; Parisi, C; Inglese, S; Temraz, TA; Nassar, HF; Guerriero, G (2020) Water 12: 2983

A two-fold integrated research study was conducted; firstly, to understand the effects of copper (Cu) and zinc (Zn) on the growth and oxidative stress in Nile tilapia, *Oreochromis niloticus*; secondly, to study the beneficial effects of the duckweed *Lemna minor* L. as a heavy metal remover in wastewater. Experiments were conducted in mesocosms with and without duckweed. Tilapia fingerlings were exposed to Cu (0.004 and 0.02 mg L^{-1}) and Zn (0.5 and 1.5 mg L^{-1}) and fish fed for four weeks. We evaluated the fish growth performance, the hepatic DNA structure using comet assay, the expression of antioxidative genes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx and glutathione-S-transferase, GST) and GPx and GST enzymatic activity. The results showed that Zn exhibited more pronounced toxic effects than Cu. A low dose of Cu did not influence the growth whereas higher doses of Cu and Zn significantly reduced the growth rate of tilapia compared to the control, but the addition of duckweed prevented weight loss. Furthermore, in the presence of a high dose of Cu and Zn, DNA damage decreased, antioxidant gene expressions and enzymatic activities increased. In conclusion, the results suggest that duckweed and Nile tilapia can be suitable candidates in metal remediation wastewater assessment programs.

Structural identification and UPLC-ESI-QTOF-MS2 analysis of flavonoids in the aquatic plant *Landoltia punctata* and their in vitro and in vivo antioxidant activities

Tsolmon, B; Fang, Y; Yang, T; Guo, L; He, K; Li, GY; Zhao, H (2020) Food Chemistry 128392

Duckweeds have long been consumed as vegetables in several South Asian countries. In this study of the chemical constituents of duckweed *Landoltia punctata*, a new compound, apigenin 6-C-[beta-D-apiofuranosyl-(12)]-beta-D-glucopyranoside (1), and a previously LC-MS identified compound, quercetin 3-O-beta-D-apiofuranoside (3), as well as three known compounds, luteolin 6-C-[beta-D-apiofuranosyl-(12)]-beta-D-glucopyranoside (2), apigenin 6-C-beta-D-glucopyranoside (4), and luteolin 7-O-neohespirodise (5), were isolated and identified on the basis of MS and NMR spectroscopic analyses and chemical derivations. In total, 24 flavonoids were identified in *L. punctata* 0001 by UPLC-ESI-QTOF-MS2. In DPPH and ABTS assays, 3 exhibited significant antioxidant activity with IC_{50} values of 4.03±1.31g/mL and 14.9±2.28g/mL, respectively. In in vivo antioxidant activity assays, 1 significantly increased the survival rate of juglone-exposed *Caenorhabditis elegans* by 2 to 3-fold, and by 75% following thermal damage. Compounds 1-5 exhibited moderate scavenging capacities of intracellular reactive oxygen species in *C. elegans* exposed to H_2O_2.

Compatibility of the invasive alien *Lemna minuta* and its potential biocontrol agent *Cataclysta lemnata*

Mariani, F; Ellwood, NTW; Zuccarello, V; Ceschin, S (2020) Water 12: 2719

The American duckweed *Lemna minuta* is invasive in freshwater habitats across much of Europe, often causing serious ecological impacts. To date, few studies have addressed how to halt its expansion. However, encouraging empirical evidence of *L. minuta* control by the aquatic herbivorous larvae of the insect *Cataclysta lemnata* is emerging. To better understand the biocontrol capacity of *C. lemnata*, information on overlap in the phenology and the growth conditions in nature of both species is fundamental. In this study, *L. minuta* and *C. lemnata* populations were analyzed in the field to define (i) their phenological features, (ii) the main environmental characteristics where the two species occur, and (iii) any overlap or difference in phenology and ecological requirements. The seasonal occurrence of the two species and environmental data were collected from 31 wetlands in central Italy. The two species showed a large phenological overlap and ecological similarities. Populations of *L. minuta* and *C. lemnata* were found all year long, although abundances were greater in spring and summer. Both species preferred waters that were shallow, circumneutral, with moderately high conductivity and trophic level and with low dissolved oxygen. The phenology and ecology of the two species were shown to be compatible, suggesting the insect could be released in natural sites invaded by the alien *L. minuta* where could act as potential biocontrol agent of it.

Molecular Biology

Identification, phylogeny, and comparative expression of the lipoxygenase gene family of the aquatic duckweed, *Spirodela polyrhiza*, during growth and in response to methyl jasmonate and salt

Upadhyay, RK; Edelman, M; Mattoo, AK (2020) International Journal of Molecular Sciences 21: DOI:10.3390/ijms21249527

Lipoxygenases (LOXs) (EC 1.13.11.12) catalyze the oxygenation of fatty acids and produce oxylipins, including the plant hormone jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA). Little information is available about the LOX gene family in aquatic plants. We identified a novel LOX gene family comprising nine LOX genes in the aquatic plant *Spirodela polyrhiza* (greater duckweed). The reduced anatomy of *S. polyrhiza* did not lead to a reduction in LOX family genes. The 13-LOX subfamily, with seven genes, predominates, while the 9-LOX subfamily is reduced to two genes, an opposite trend from known LOX families of other plant species. As the 13-LOX subfamily is associated with the synthesis of JA/MeJA, its predominance in the *Spirodela* genome raises the possibility of a higher requirement for the hormone in the aquatic plant. JA-/MeJA-based feedback

regulation during culture aging as well as the induction of LOX gene family members within 6 h of salt exposure are demonstrated.

Physiology & Stress

Effects of artificial ultraviolet B radiation on the macrophyte *Lemna minor*: a conceptual study for toxicity pathway characterization

Xie, L; Solhaug, KA; Song, Y; Johnsen, B; Olsen, JE; Tollefsen, KE (2020) Planta 252: 86.

Main conclusion UVB radiation caused irradiance-dependent and target-specific responses in non-UVB acclimated *Lemna minor*. Conceptual toxicity pathways were developed to propose causal relationships between UVB-mediated effects at multiple levels of biological organisation. Macrophytes inhabit waterways around the world and are used in hydroponics or aquaponics for different purposes such as feed and wastewater treatment and are thus exposed to elevated levels of UVB from natural and artificial sources. Although high UVB levels are harmful to macrophytes, mechanistic understanding of irradiance-dependent effects and associated modes of action in non-UVB acclimated plants still remains low. The present study was conducted to characterise the irradiance-dependent mechanisms of UVB leading to growth inhibition in *Lemna minor* as an aquatic macrophyte model. The *L. minor* were continuously exposed to UVB (0.008-4.2 W m^{-2}) and constant UVA (4 W m^{-2}) and photosynthetically active radiation, PAR (80 µmol m^{-2} s^{-1}) for 7 days. A suite of bioassays was deployed to assess effects on oxidative stress, photosynthesis, DNA damage, and transcription of antioxidant biosynthesis, DNA repair, programmed cell death, pigment metabolism and respiration. The results showed that UVB triggered both irradiance-dependent and target-specific effects at multiple levels of biological organization, whereas exposure to UVA alone did not cause any effects. Inhibition of photosystem II and induction of carotenoids were observed at 0.23 W m^{-2}, whereas growth inhibition, excessive reactive oxygen species, lipid peroxidation, cyclobutane pyrimidine dimer formation, mitochondrial membrane potential reduction and chlorophyll depletion were observed at 0.5 - 1 W m^{-2}. Relationships between responses at different levels of biological organization were used to establish a putative network of toxicity pathways to improve our understanding of UVB effects in aquatic macrophytes under continuous UVB exposures. Additional studies under natural illuminations were proposed to assess whether these putative toxicity pathways may also be relevant for more ecologically relevant exposure scenarios.

Effects of low and high irradiation levels on growth and PSII efficiency in *Lemna minor* L.

Lepedus, H; Vidakovic-Cifrek, Z; Sebalj, I; Dunic, JA; Cesar, V (2020) Acta Botanica Croatica 79: 185-192

Plant growth and reproduction depend on light energy that drives photosynthesis. In the present study we compared growth characteristics, photosynthetic pigments content and photosystem II (PSII) performance in *Lemna minor* L. grown in two different irradiation regimes: low light (LL) -50 µmol(PHOTONS) m^{-2} s^{-1} and high light (HL) -500 µmol (PHOTONS) m^{-2} s^{-1}. The main goal was to investigate the photosynthetic regulatory mechanisms that ensure adjustment to different light conditions and integrate these observations with the data on plant multiplication and biomass production. For this purpose, we measured chlorophyll (Chls) and carotenoid (Cars) contents and analyzed the energy fluxes through the PSII by saturation pulse method as well as by Chl a transient induction and JIP test. In a comparison of the effect of LL and HL on plant multiplication and fresh biomass, it was shown that the effect on growth was primarily attributed to the biomass reduction in LL while the effect on number of plants was much smaller. Total Chl and Cars contents were decreased in plants exposed to HL which indicated long-term acclimation response to the increased

irradiance. Furthermore, the HL plants revealed better capability for the utilization of absorbed light in photosynthesis accompanied by photoprotective adjustment of certain number of PSII reaction centers from active to dissipative mode of functioning. In conclusion, our study showed that duckweed plants had great adjustment potential to different irradiation conditions, which might be of great importance not only under variable light availability but also when simultaneously challenged by some other environmental disturbance (e.g. different pollutants).

Lemna minor studies under various storage periods using extended-polarity extraction and metabolite non-target screening analysis

Wahman, R; Grassmann, J; Sauvetre, A; Schroder, P; Letzel, T (2020) Journal of Pharmaceutical and Biomedical Analysis 188: 113362

Plant metabolomic studies cover a broad band of compounds, including various functional groups with different polarities and other physiochemical properties. For this reason, specific optimized methods are needed in order to enable efficient and non-destructive extraction of molecules over a large range of LogD values. This study presents a simple and efficient extraction procedure for *Lemna minor* samples demonstrating polarity extension of the molecular range. The *Lemna* samples chosen were kept under the following storage conditions: 1) fresh, 2) stored for a few days at -80 degrees C, and 3) stored for 6 months at -80 degrees C. The samples were extracted using five specifically chosen solvents: 100 % ethanol, 100 % methanol (MeOH), acidic 90 % MeOH (MeOH-water-formic acid (FAC) (90:9.5:0.5, v/v/v), MeOH-water (50:50, v/v), and 100 % water. The final extraction procedure was conducted subject to three solvent conditions, and the subsequent polarity-extended analysis was applied for *Lemna minor* samples using RPLC-HILIC-ESI-TOF-MS. The extraction yield is in descending order (acidic 90 % MeOH), 50 % MeOH, 100 % water and 100 % MeOH. The results displayed significant molecular differences, both in the extracts investigated and in the fresh *Lemna* samples, compared to stored samples, in terms of the extraction yield and reducing contents as well as the number of features. The storage of *Lemna minor* resulted in changes to the fingerprint of its metabolites as the reducing contents increased. The comparisons enable a direct view of molecule characterizations, in terms of their polarity, molecular mass, and signal intensity. This parametric information would appear ideal for further statistical data analysis. Consequently, the extraction procedure and the analysis/data evaluation are highly suitable for the so-called extended-polarity non-target screening procedure.

The molecular mechanism underlying cadmium resistance in NHX1 transgenic *Lemna turionifera* was studied by comparative transcriptome analysis

Yao, J; Sun, JG; Chen, YK; Shi, LQ; Yang, L; Wang, Y (2020) Plant Cell Tissue and Organ Culture 143: 189-200

Comparative transcriptome analysis was applied to study the involvement of NHX1 in cadmium detoxification. The genes associated with root, calcium signal and cadmium transports were regulated in transgenic duckweed. Cadmium (Cd) is one of the most toxic heavy metals, which poses a serious threat to the environment. Our previous studies revealed that transgenic *Lemna turionifera* (hereafter mentioned as Transgenic) over expressing Na^+/H^+ Antiporter (AtNHX1) gene was more tolerant to Cd^{2+} stress than the Wild type plants (WT). To further explore the molecular mechanism underlying cadmium resistance, comprehensive transcriptome analysis and comparison were performed. For RNA-Seq, the trannsgenic and WT were treated with 50 μM $CdCl_2$ for 24 h. In total, 2247 differentially expressed unigenes (DEGs) including 1195 upregulated and 1052 downregulated ones were discovered between Transgenic and WT samples. The downregulated genes associated with the adventitious root (AR), lateral root (LR) and root hair (RH) initiation might have prevented the root from abscission. Calcium signaling and ROS accumulation were regulated in

response to cadmium stress. Moreover, the activation of genes involved in the production of trehalose and R-S glutathione increased the cadmium resistance of duckweed. Downregulation of genes involved in the transport of Cd^{2+} and upregulation of genes involved in vacuolar sequestration might have resulted in increased tolerance of Transgenic over WT. These findings might provide new ideas for improving plant cadmium resistance and phytoremediation.

Phytoremediation

Differential bioaccumulation of select heavy metals from wastewater by *Lemna minor*

Khan, MA; Wani, GA; Majid, H; Ul Farooq, F; Reshi, ZA; Husaini, AM; Shah, MA (2020) Bulletin of Environmental Contamination and Toxicology 105: 777-783

The capacity of *Lemna minor* to remediate toxic heavy metals from wastewater is reasonably well documented. In view of the pivotal role of this species in the environmental clean-up, here we evaluated the bioaccumulation potential of *L. minor* for cadmium (Cd), lead (Pb), and nickel (Ni) through a controlled experiment. *L. minor* tolerated the metals Cd, Ni, and Pb up to 0.5, 5, and 8 mg/L, respectively, and beyond these concentrations the toxicity symptoms appeared. Bio-concentration factor varied at different concentrations of heavy metals tested. Overall, *L. minor* showed good phytoremediation potential for all the three tested heavy metals (Cd, Ni, and Pb), though in relative terms it was more effective in extracting Ni and Cd, as compared to Pb, both in single and mixed concentrations. In view of the growing pollution in Kashmir Himalayan aquatic habitats the phytoremediation by invasive species such as *L. minor* promises to be one of the best choices than other native plants for cleaning up of polluted soils/water because of its fast growth rate, high abundance, easy handling, and wide distribution in Kashmir Himalayan aquatic ecosystems.

Application of biochars obtained through the pyrolysis of *Lemna minor* in the treatment of Ni-electroplating wastewater

Yan, FL; Wang, Y; Wang, WH; Zhao, JX; Feng, LL; Li, JJ; Zhao, JC (2020) Journal of Water Process Engineering 37: 101464

A feasible approach was studied for utilizing the harvested *Lemna minor* to treat Ni-Electroplating Waste Water (NEWW). The *Lemna minor* was pyrolyzed under oxygen-limited condition at various temperatures to produce biochar firstly and the optimal biochar was selected according to the yield of biochars and the adsorption capacity of biochars to treat the simulated Ni-containing wastewater. A combination method of pre-precipitation and biochar adsorption was proposed and validated by treatment of an actual NEWW. Results indicated that the biochar fabricated from *Lemna minor* at 400°C (LM400) was the optimal biochar for Ni^{2+} adsorption and the adsorption capacity of LM400 was 41.68 mg/g. Precipitation with 5.0 g/L $Ca(OH)_2$ succeeded by two-step adsorption with LM400 could yield qualified treatment streams when the actual NEWW was treated. Moreover, the adsorption mechanism of biochars to Ni^{2+} could mainly be ascribed to the complexation and ion-exchange reactions of Ni^{2+} in wastewater with the oxygen-containing functional groups and K^+ ions of the biochar according to the results of FTIR and XRD. This study shows that *Lemna minor* is a viable raw material to produce strong adsorption biochar, which could be used as excellent adsorbents for heavy metals, such as Ni, Cu and Zn. Meanwhile, the actual NEWW treated with the combined process of two steps could meet discharge requirements.

Multifaceted roles of duckweed in aquatic phytoremediation and bioproducts synthesis

Liu, Y; Xu, H; Yu, CJ; Zhou, GK (2020) Global Change Biology Bioenergy 13: 70-82.

Duckweed (Lemnaceae) is a fast-growing aquatic vascular plant. It has drawn an increasing attention worldwide due to its application in value-added nutritional products and in sewage disposal. In particular, duckweed is a promising feedstock for bioenergy production. In this review, we summarized applications of duckweed from the following four aspects. Firstly, duckweed could utilize nitrogen, phosphorus, and inorganic nutrition in wastewater and reduces water eutrophication efficiently. During these processes, microorganisms play an important role in promoting duckweed growth and improving its tolerance to stresses. We also introduced our pilot-scale test using duckweed for wastewater treatment and biomass production simultaneously. Secondly, its capability of fast accumulation of large amounts of starch makes duckweed a promising bioenergy feedstock, catering the currently increasing demand for bioethanol production. Pretreatment conditions prior to fermentation can be optimized to improve the conversion efficiency from starch to bioethanol. Furthermore, duckweed serves as an ideal source for food supply or animal feed because the composition of amino acids in duckweed is similar to that of whey protein, which is easily digested and assimilated by human and other animals. Finally, severing as a natural plant factory, duckweed has shown great potential in the production of pharmaceuticals and dietary supplements. With the surge of omics data and the development of Clustered Regularly Interspaced Short Palindromic Repeats technology, remodeling of the metabolic pathway in duckweed for synthetic biology study will be attainable in the future.

Phytotoxicity

Chemical forms governing Cd tolerance and detoxification in duckweed (*Landoltia punctata*)

Wang, XL; Zhang, BJ; Wu, DS; Hu, L; Huang, T; Gao, GQ; Huang, S; Wu, S (2021) Ecotoxicology and Environmental Safety 207: 111553

Duckweed (*Landoltia punctata*) is an ideal species to restore cadmium (Cd)-polluted waters due to its fast growth and easy harvesting. To understand its tolerance and detoxification mechanism, the Cd stress responses, sub-cellular Cd distribution and chemically bound Cd forms (especially protein-bound Cd) were surveyed in this study. *L. punctata*, a potential Cd bioremediation plant, was cultured hydroponically with Cd concentrations of 0.0, 0.5, 2.0, and 5.0 mg L^{-1} for 5 days. The results showed that the Cd content in *L. punctata* increased significantly as the Cd content increased. The majority of Cd was localized in the soluble fraction (23-55%) and the cell wall fraction (21-54%), and only 14-23% of Cd was located in cell organelles. Analysis of the Cd chemical forms demonstrated that the largest portion of Cd was found in 1 M NaCl extracts, followed by d-H_2O and 2% HAc extracts, indicating that Cd was mainly bound to different proteins. Albumin and globulin-bound Cd forms were predominant, together accounting for over 80% of the total protein-bound Cd in *L. punctata*. These results indicate that cell wall immobilization and vacuolar dissociation of Cd are possible primary strategies for Cd biosorption and detoxification in *L. punctata*, which occur mainly through chemical forms changes, especially the binding of Cd to proteins.

Joint effects of naphthalene and microcystin-LR on physiological responses and toxin bioaccumulation of *Landoltia punctata*

Yang, GLi; Huang, MJ; Tan, A; Lv, SM (2020) Aquatic Toxicology 231:105710

The co-contamination of naphthalene (NAP) and microcystin-LR (MC-LR) commonly occurs in eutrophic waters. However, the joint effects of NAP and MC-LR on plants in aquatic environments remain unknown. *Landoltia punctata* is characterized by high starch yields and high biomass in polluted waters and has been proven to be a bioenergy crop and phytoremediation plant. In this study, *L. punctata* was cultured in a nutrient medium with environmentally relevant NAP (0.1, 1, 3, 5, and 10 μg/L) and MC-LR (5, 10, 25, 50, and 100 μg/L) to determine individual and joint toxic effects. The effects of NAP and MC-LR on physiological responses of *L. punctata*, including growth, starch accumulation, and antioxidant responses, were studied. Bioaccumulation of MC-LR in *L. punctata*, with or without NAP, was also examined. The results showed that growth and chlorophyll-a contents of *L. punctata* were reduced at high concentrations of MC-LR (≥ 25 μg/L), NAP (≥ 10 μg/L) and their mixture (≥ 10 ± 1 μg/L) after exposure for 7 d. Starch accumulation in *L. punctata* did not decrease when exposed to NAP and MC-LR, and higher starch content of 29.8 % ± 2.7 % DW could be due to the destruction of starch-degrading enzymes. The antioxidant responses of *L. punctata* were stronger after exposure to MC-LR + NAP than when exposed to a single pollutant, although not enough to avoid oxidative damage. NAP enhanced the bioaccumulation of MC-LR in *L. punctata* when NAP concentration was higher than 5 μg/L, suggesting that higher potentials of MC-LR phytoremediation with *L. punctata* may be observed in NAP and MC-LR co-concomitant waters. This study provides theoretical support for the application of duckweed in eutrophic waters containing organic chemical pollutants.

Enhanced Cd accumulation by Graphene oxide (GO) under Cd stress in duckweed

Yang, L; Chen, Y; Shi, L; Yu, J; Yao, J; Sun, J; Zhao, L; Sun, J (2020) Aquatic Toxicology 229:105579

Effective phytoremediation by aquatic plant such as duckweed could be applied to solve Cd pollution. In the present study, the impact of Graphene oxide (GO) on the accumulation of Cd in duckweed has been studied. The response of duckweed was also investigated, concluding growth, Cd^{2+} flux, and gene expression response. Results showed that GO promoted the accumulation of Cd in duckweed. After 6h of Cd enrichment in duckweed, Cd content was about 1.4 times that of the control group at fronds and 1.25 times that of the control group at roots, meanwhile, Cd content in the water system was 0.67 times that of the control group. The Cd^{2+} influx increased significantly. 4471 genes were up-regulated and 3230 genes were down-regulated significantly as duckweed treated with GO under Cd treatment. Moreover, phagosome pathway was downregulated, some key proteins: Stx7, Rab7 and Tubastatin B (TUBB) were significantly downregulated with GO addition under Cd stress. Scanning electron microscope (SEM) observation showed that GO and Cd were attached on the cell surface of duckweed as white crystal. GO could be applied in phytoremediation by duckweed of Cd in aquatic system.

Combined toxicity of therapeutic pharmaceuticals to duckweed, *Lemna minor*

Markovic, M; Neale, PA; Nidumolu, B; Kumar, A (2020) Ecotoxicology and Environmental Safety 208:111428

Pharmaceuticals, which are designed to be biologically active at low concentrations, are found in surface waters, meaning aquatic organisms can be exposed to complex mixtures of pharmaceuticals. In this study, the adverse effects of four pharmaceuticals, 17alpha-ethynylestradiol (synthetic estrogen), methotrexate (anticancer drug), diclofenac (nonsteroidal anti-inflammatory drug) and fluoxetine (antidepressant), and their binary mixtures at mg/L concentrations were assessed using the 7-day *Lemna minor* test, with both apical and biochemical markers evaluated. The studied biochemical markers included chlorophyll a, chlorophyll b, carotenoids and oxidative stress enzymes catalase, glutathione-S-transferase and glutathione reductase, with effects compared to solvent controls. The adverse effects on *Lemna minor* were dose-dependent for frond

number, surface area, relative chlorophyll content and activity of glutathione S-transferase for both individual pharmaceuticals and binary mixtures. According to the individual toxicity values, all tested pharmaceuticals can be considered as toxic or harmful to aquatic organisms, with methotrexate considered highly toxic. The most sensitive endpoints for the binary mixtures were photosynthetic pigments and frond surface area, with effects observed in the low mg/L concentration range. The concentration addition model and toxic unit approach gave similar mixture toxicity predictions, with binary mixtures of methotrexate and fluoxetine or methotrexate and 17alpha-ethynylestradiol exhibiting synergistic effects. In contrast, mixtures of diclofenac with fluoxetine, 17alpha-ethynylestradiol or methotrexate mostly showed additive effects. While low concentrations of methotrexate are expected in surface water, chronic ecotoxicological data for invertebrates and fish are lacking, but this is required to better assess the environmental risk of methotrexate.

Pulsed exposure of the macrophyte *Lemna minor* to herbicides and the mayfly Neocloeon triangulifer to diamide insecticides

Sanford, M; Washuck, N; Carr, K; Prosser, RS (2020) Chemosphere 128582.

Pesticides applied to agricultural land can enter aquatic ecosystems through runoff or leaching during precipitation events. In a lotic system, these events result in a pulse of exposure to biota living in these systems. The concentration of pesticide increases, peaks, and then gradually declines, and this pulsed exposure may occur multiple times over the course of a growing season. The dynamic nature of exposure to pesticides in the environment is not often mimicked in the laboratory testing of the toxicity of pesticides. The present study investigated the potential latent effects of a 24-h pulsed exposure of metolachlor, metribuzin, MCPA (2-methyl-4-chlorophenoxyacetic acid), MCPP (methylchlorophenoxypropionic acid or mecoprop), dicamba, and 2,4-D to the aquatic macrophyte *Lemna minor* followed by a 5-day recovery period. The relative sensitivity of *L. minor* to the herbicides were, in this decreasing order: metolachlor>metribuzin >2,4-D>MCPA>MCPP>dicamba. This study also investigated the effects of short-term exposures of the diamide insecticides cyantraniliprole and chlorantraniliprole on the survival of the larvae of the parthenogenetic mayfly Neocloeon triangulifer. The median lethal concentrations (96-h LC_{50}s) for cyantraniliprole and chlorantraniliprole were 8.60 and 2.92 µg/L, respectively.

Single and binary equilibrium studies for Ni^{2+} and Zn^{2+} biosorption onto *Lemna gibba* from aqueous solutions

Morales-Barrera, L; Flores-Ortiz, CM; Cristiani-Urbina, E (2020) Process 8: 1089

The biosorption ability of *Lemna gibba* for removing Ni^{2+} and Zn^{2+} ions in aqueous batch systems, both individually and simultaneously, was examined. The influences of solution pH and initial single and binary metal concentrations on equilibrium Ni^{2+} and Zn^{2+} biosorption was explored. The optimal solution pH for Ni^{2+} and Zn^{2+} biosorption was 6.0, for both the single and binary metal systems. Ni^{2+} and Zn^{2+} biosorption capacities increased with increasing initial metal concentrations. The presence of Zn^{2+} ions more adversely affected the biosorption of Ni^{2+} ions in the binary metal systems than vice versa. The single and binary biosorption isotherms of Ni^{2+} and Zn^{2+} revealed that *L. gibba*'s affinity for Zn^{2+} ions was higher than that for Ni^{2+} ions. The Redlich-Peterson and Freundlich isotherm models fit well to the experimental equilibrium data of Ni^{2+} ions, whereas Redlich-Peterson and Langmuir models better described the equilibrium data of Zn^{2+} ions in single metal systems. The modified Sips isotherm model best fit the competitive biosorption data of Ni^{2+}-Zn^{2+} on *L. gibba*. FTIR analyses suggest the involvement of hemicellulose and cellulose in the biosorption of Ni^{2+} and Zn^{2+}. The presence of Ni^{2+} and Zn^{2+} on the *L. gibba* surface was validated by SEM-EDX.

Reviews

Duckweed: Biological Chemistry and Applications

Edelman, M; Appenroth, KJ; Sree, KS (2020) Frontiers in Sustainable Food Systems 4: 615135

As its name implies, duckweeds are a favorite food source for fowl, several fish and other animals as well (Appenroth et al., 2015). Under optimal conditions in nature or in the laboratory, several duckweed species can double their biomass almost daily representing the fastest growing Angiosperms. Depending on the cultivation conditions, the protein content of the biomass may reach up to 40% or more of the dry weight or the biomass may accumulate starch up to 50 % of the dry weight. In controlled conditions, they can be grown axenically, either autotrophically, mixotrophically, or even heterotrophically (Landolt and Kandeler, 1987). In addition, the genomes of some duckweeds (e.g., *S. polyrhiza*; genome size,160 ± 3 Mbp/1C) are among the smallest for a higher plant (Wang et al., 2011; Bog et al., 2015). Presently, the genome sequence of *S. polyrhiza* clone 9,509 represents the "gold standard" for duckweed genomes (Hoang et al., 2018). Coupled with the increasing abilities of several groups to genetically transform (Vunsh et al., 2007) various species of this aquatic family (recently, by CRISPR-Cas, Liu et al., 2019), one can think of "upcoming model system" or "biotech applications."

Instructions to Contributors for the Duckweed Forum

The Duckweed Forum (DF) is an electronic publication that is dedicated to serve the Duckweed Research and Applications community by disseminating pertinent information related to community standards, current and future events, as well as other commentaries that could benefit this field. As such, involvement of the community is essential and the DF can provide a convenient platform for members in the field to exchange ideas and observations. While we would invite everyone to contribute, we do have to establish clear guidelines for interested contributors to follow in order to standardize the workflow for their review and publication by the Duckweed Steering Committee members.

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.
DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 1,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

In addition to invitees by a Duckweed Steering Committee member, if you are considering submitting a contribution to DF but are unsure about the fit of your idea, please feel free to contact one of the members in the Duckweed Steering Committee in order to obtain feedback as to the appropriateness of the subject for DF. Please include a few sentences describing the overall topic that you are interested to present on, and why you think it is of interest to the general duckweed community. If you have the abstract or draft text prepared, please include it. The Duckweed Steering Committee will discuss the material in one of its meetings and the decision to formally invite submission will be given shortly afterwards.

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All listed authors must concur in the submission and the final version must be seen and approved by all authors of the contribution. As a public forum, we do not carry out any Copyright application. If you need to copyright your material, please do so beforehand.

Formatting requirements:

- A commonly used word processing program, such as Word, is highly recommended.

- Formatting requirements: 8.5-by-11-inch (or 22 cm-by-28 cm) paper size (standard US letter).
- Single-spaced text throughout.
- One-inch (or 2.5 cm) left and right, as well as top and bottom margins.
- 11-point Times New Roman font.
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Title:

- Should be intelligible to DF readers who are not specialists in the field and should convey your essential points clearly.
- Should be short (no more than 150 characters including spaces) and informative.
- Should avoid acronyms or abbreviations aside from the most common biochemical abbreviations (e.g., ATP). Other acronyms or abbreviations should either:
    - be introduced in their full form (e.g., Visualization of Polarized Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) Activity in Live Cells by Fluorescence Resonance Energy Transfer (FRET) Imaging); or
    - be clarified by use as a modifier of the appropriate noun (e.g., FOX1 transcription factor, ACC dopamine receptor).

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It is extremely important that figures be prepared with the proper resolution for publication in order to avoid inaccurate presentation of the data. The minimum acceptable resolution for all figures is 300 dpi. Excessive file compression can distort images, so files should be carefully checked after compression. Note that figures that contain both line art (such as graphs) and RGB/grayscale areas (such as photographs) are best prepared as EPS (vector) files with embedded TIFF images for the RGB/grayscale portions. The resolution of those embedded TIFF images should be at least 300 dpi. Original images should be submitted as a separate file to the text file. It would be helpful to insert the intended into the Word file as well, if desired, to indicate the location for it. The legend to the image/figure should be added at the end of the text file and labeled as "Legend to Figures".

Links for Further Reading

http://www.ruduckweed.org/ Rutgers Duckweed Stock Cooperative, New Brunswick, New Jersey State University. Prof. Dr. Eric Lam

http://www.InternationalLemnaAssociation.org/ Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants.

Community Resources - Updated Table for Duckweed Collections in the Community

For information related to the location, collection size and contact email for duckweed collections in our community, please access the website of the RDSC (Rutgers Duckweed Stock Cooperative) under the heading "List of Worldwide Duckweed Collections". This Table will be updated as new entries for duckweed collections are being supplied to members of the International Steering Committee for Duckweed Research and Applications (ISCDRA). We also plan to publish the updated table in the first issue of each Duckweed Forum newsletter volume starting in 2021.

Note to the Reader

Know of someone who would like to receive their own copy of this newsletter? Would you like to offer ideas for future articles or have comments about this newsletter? Need to be added or removed from our contact list?

Please let us know via email to the Chair of ISCDRA, Prof. Eric Lam: ericL89@hotmail.com